ON CHEMISTRY AND FUNCTION OF COENZYME A[1]

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In recent years, the nutritional importance of the vitamins has been amplified by the understanding of their metabolic function. It appears rather clearly that every vitamin functions in what may be called a distinct metabolic territory. It is shaped for a special metabolic function by its particular chemical nature. In return, the identification of this special functioning of the various chemical individuals which we call "vitamins" has recently shed considerable light into many metabolic processes.

Specifically, with pantothenic acid, we have been able, during the last half-dozen years, to amplify the pioneer work of Roger Williams (74, 75). We have found a specific metabolic territory for the pantothenic acid through, I would say, a number of fortunate accidents. During the study of a relatively simple, so-called "detoxication reaction", the acetylation of aromatic amines in liver extracts, we observed the need of a heat-stable cofactor (36, *cf.* also 18, 48) which did not seem to coincide with any then known coenzyme. Eventually, on isolation of this new coenzyme, it was found to contain pantothenic acid (21). The discovery of pantothenic acid in the coenzyme, to which we gave the name "coenzyme A (CoA)", was greatly facilitated through help from Dr. Roger Williams' laboratory, in particular, by Dr. Beverly Guirard. She analyzed the material which we submitted, and it was through her skilful observations that pantothenic acid was detected in the coenzyme (21, 38). When bound in CoA, pantothenic acid appeared unavailable to most test organisms, including the most commonly used, *Lactobacillus arabinosus*. To identify it, Guirard hydrolyzed the coenzyme with acid and determined β-alanine by yeast growth. These observations prompted us to develop a method for the liberation of pantothenic acid from CoA by a double enzymic treatment, namely, with intestinal phosphatase and with an initially unidentified liver enzyme (51).

The thus established connection of pantothenic acid with our seemingly off-the-road acetylation reaction was the clue that we were dealing here with a phenomenon of primary metabolic importance. On the other hand, isotope studies had demonstrated amply that a so far rather mysterious metabolic unit called "active acetate" or "active 2-carbon residue" was an intermediary which operated as a building stone in a large number of synthetic reactions (5, 6). When, soon after the discovery of CoA, a great variety of acetylation reactions ap-

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peared to be CoA dependent (52, 53, 59, 68), it was not too difficult to reach the conclusion that with this coenzyme we were dealing with the transfer system for the mysterious "active acetate" (31a). Recent developments have now led to a precise chemical formulation of the acetyl carrier function of CoA (43).

An important lead towards eventual understanding of this carrier function has been an old observation, namely, that in addition to pantothenic acid, CoA contained a sulfur compound (17, 37, 38). We early observed that sulfur was present as a disulfide in the isolated coenzyme. After reduction with cyanide, we found that a strong nitroprusside reaction for SH-groups was given by all our coenzyme preparations.

I must confess that initially we did not pay enough attention to the sulfur because we were so engrossed by the presence of the vitamin pantothenic acid. However, on progressive purification, the sulfur content increased parallel with the purity of the coenzyme (17), and it became certain that the sulfur was part of it. This observation actually became the lead for Lynen (43) to suspect that the acetyl in acetyl-CoA may be bound as a mercaptoester.

Our interest in the sulfur was greatly stimulated by the discovery by Williams, Hoff-Jørgensen, and Snell (76) of a growth factor for *Lactobacillus bulgaricus*, originally named "LBF", and now known as "pantethine" (7, 59). This they found to be a component of CoA. Intact CoA was found to be inactive, but LBF was liberated from CoA on dephosphorylation. Furthermore, they could inactivate LBF by treatment with the liver enzyme, which earlier had been found by us to be essential for the liberation of pantothenic acid from CoA, in addition to intestinal phosphatase. We then found that on dephosphorylation of CoA, a pantothenic acid-sulfur combination appeared (17) which could be spotted chromatographically by cyanide-nitroprusside spray at the same level as an LBF preparation which had kindly been supplied to us by Dr. W. L. Williams. This is shown in figure 1. At the same time the LBF preparation, just as CoA, was found strongly nitroprusside positive after cyanide treatment. Chromatography of acid hydrolyzates of CoA suggested that the sulfur compound should be bound to the carboxyl group of pantothenic acid (17). This observation, prompted by the discovery of the new growth factor by Snell and his group (59), in one stroke unravelled for us two mysteries of long standing: (a) the function of the liver enzyme in the liberation of pantothenic acid, and (b) the fitting of the sulfur compound into the structure of the coenzyme. Meanwhile, Snell, Brown, and their group (59) had gone further with the LBF analysis and, in a brilliant spurt of work, had identified the factor as a "dipeptide" with a peptidic link between the carboxyl group of pantothenic acid and the amino group of thioethanolamine (= decarboxylated cysteine, or cysteamine). By analysis and synthesis, they identified through this work the sulfur component of CoA as thioethanolamine. A simplified and rather convenient procedure for the synthesis of pantethine was described more recently by Baddiley (2).

Most recently, as has been already mentioned, a further important amplification of the role of the sulfur in the coenzyme was brought out by the work of Lynen and Reichert (43, 44). They isolated acetyl-CoA from yeast and could

identify it as acetyl mercapto-CoA. This makes the sulfhydryl group the functional group which shuttles back and forth from free —SH to —S—acetyl. The functioning of CoA in the reduced, sulfhydryl form now explains the early observed need for cysteine or glutathione in all CoA linked reactions. The pantothenic acid part, however, of the molecule acts rather as an auxiliary or modifier; indeed, the true chemical meaning of its presence in the CoA molecule is as yet beyond our comprehension.

On the other hand, from the preparative point of view, the recognition of CoA as a compound which easily switches back and forth from —S—S— to —SH gave us some clues for the understanding of the insistent inhomogeneity

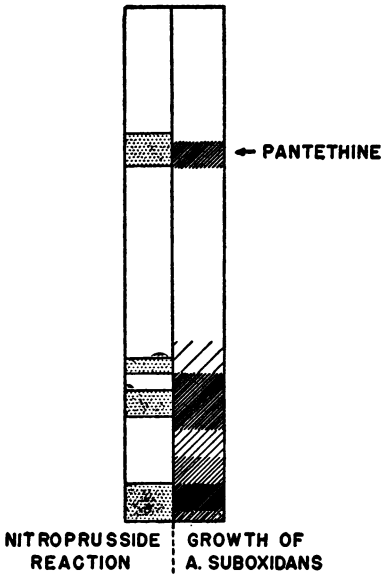

FIG. 1. Chromatogram of intestinal phosphatase-treated CoA. The most interesting is the fast moving component, which was nitroprusside positive, contained pantothenic acid, but no adenylic acid, practically identically with LBF. The more slowly moving fragments showing nitroprusside and pantothenic acid activity contained adenylic acid, representing in part, at least, partial degradation products. (*Cf.* 17.)

of our preparations (20). It appeared that the —SH group in crude extracts would cross link through oxidation with other SH-compounds as cysteine or glutathione. Analagous observations were made by Snell's group on LBF (8). We realized, therefore, that an important step in the final purification would be a complete reduction of the coenzyme to eliminate accessory groups brought in by cross-linking. In such a manner, Dr. Gregory has obtained now a nearly pure preparation of a level of 400 units per milligram (21). The composition of the preparation is shown in table 1.

A new convenient method for the preparation of CoA was recently described by Beinert *et al.* (4), based on a precipitation with glutathione-cuprous oxide. A

CoA preparation of high purity prepared in this manner is now available from the Pabst Laboratories, 1037 McKinley Avenue, Milwaukee 3, Wisconsin.

As is illustrated in figure 2, we can give a quite precise picture of the structure of CoA, which, as we very early recognized, contained adenylic acid (21) and, as Dr. Novelli has found recently, contains altogether 3 moles of phosphorus (50). A pyrophosphate bridge appears to crosslink from adenylic-5′-position to pantothenic acid-4′-position (1); the adenylic acid furthermore carries a third phosphate in monoester link. This link, according to recent work by Kaplan

TABLE 1

Composition of best preparation of CoA (21)

	CALCD. %*	FOUND %	RATIO
Pantothenic acid	28.6	26.8 (enzymatic assay) 25.6 (microbiological)	1
Adenine	17.6	17.0 (spectrophotometric)	1.05
Phosphorus (total)	12.12	10.6	2.83
Monoester phosphorus†	—	3.6	0.96
Sulfur	4.18	4.13	1.07

* Pantothenic acid, 2-mercaptoethylamine, 3 phosphoric acid, adenosine, $-5H_2O$; molecular weight 767.

† Liberated by prostate phosphomonoesterase.

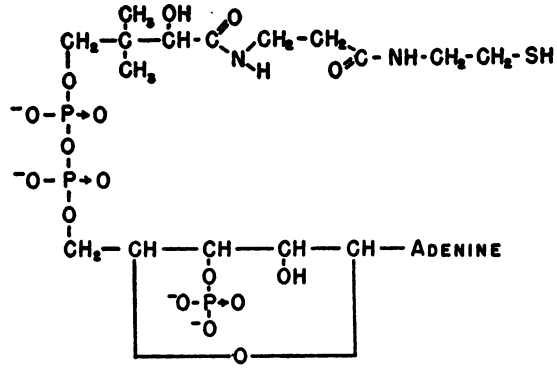

FIG. 2. Constitution of Coenzyme A.

(73), corresponds to the *b*-series of nucleotides (16) in contrast to TPN, where it corresponds to the *a*-series.

Recent studies on the enzymatic synthesis of CoA (31) have further confirmed the proposed structure. In particular, it was found that synthetic 4′-phosphopantethine, prepared by Baddiley is, in the presence of ATP, converted to CoA with the liberation of inorganic pyrophosphate (2a).

Turning now again to the acetyl transfer function of the coenzyme, the brilliant work of Lynen *et al.* (43, 44) on the isolation and identification of acetyl-CoA gave an important amplification to a scheme of acetyl transfer reactions, which we had developed from enzyme studies (13, 14, 31a). A great step for-

ward toward such a transfer scheme was obtained through the separation of the various acetyl *acceptor* systems from each other and, from the acetyl *donor* system, the so-called "ATP-acetate reaction". This separation was accomplished by a fractionation of pigeon liver extract, our main experimental tool, which is a real storehouse of acetyl transfer reactions (14). This extract contains a great variety of acceptor functions which, we were convinced, corresponded to separate units that we should be able to pull apart. Acetone fractionation appeared to be a convenient method, and the first and most encouraging result was that we could divide our sulfanilamide acetylation system into two protein fractions, inactive by themselves, but active when combined as shown in figure 3.

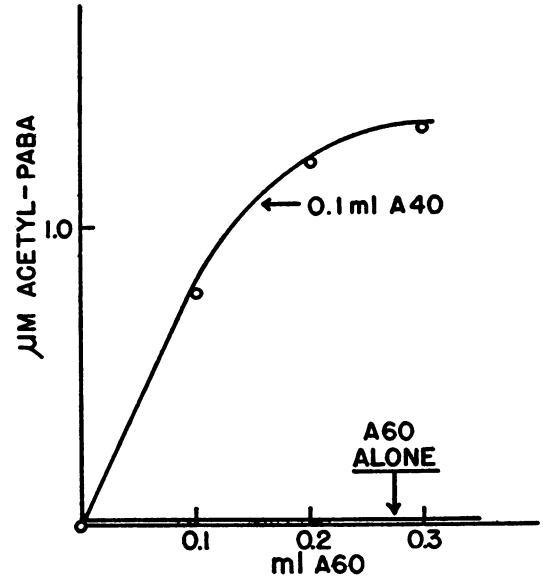

FIG. 3. Separation of the acetylation system for aromatic amines into donor and acceptor fractions. The 40 per cent acetone precipitate (A 40) contains the separated enzyme for the ATP-CoA-acetate reaction. The fraction coming out between 40 and 60 per cent acetone (A 60) contains the acceptor enzyme, the acetokinase p-Aminobenzoate (PABA) was used as acceptor. (See also 13, 15.)

The 40 per cent acetone precipitate was identified as the acetyl *donor* system, the ATP-CoA-acetate reaction, whereas the 60 per cent acetone fraction contained the *acceptor* enzyme, specific for aromatic amines. This initial success of separating the donor and acceptor fraction of our original test system led to a further fractionation, the scheme of which is shown in figure 4. The most important result is that by the use of various fractionation methods it was possible to establish the various acceptor enzymes as separate units, *e.g.*, for acetoacetate synthesis, for citric acid synthesis (13, 68, 69), and for various amine acetylations, including glucosamine (15). The acceptor enzyme for citric acid synthesis has previously been isolated and crystallized from heart muscle by Stern and Ochoa (54) and is now generally referred to as the "condensing enzyme".

We turn now to the acetyl donor systems. This study received a great impulse when Novelli made a then rather startling observation (53), which implicated our old favorite, acetyl phosphate (33). Up to then we had been unable to fit acetyl phosphate reasonably into the acetyl transfer reaction although originally

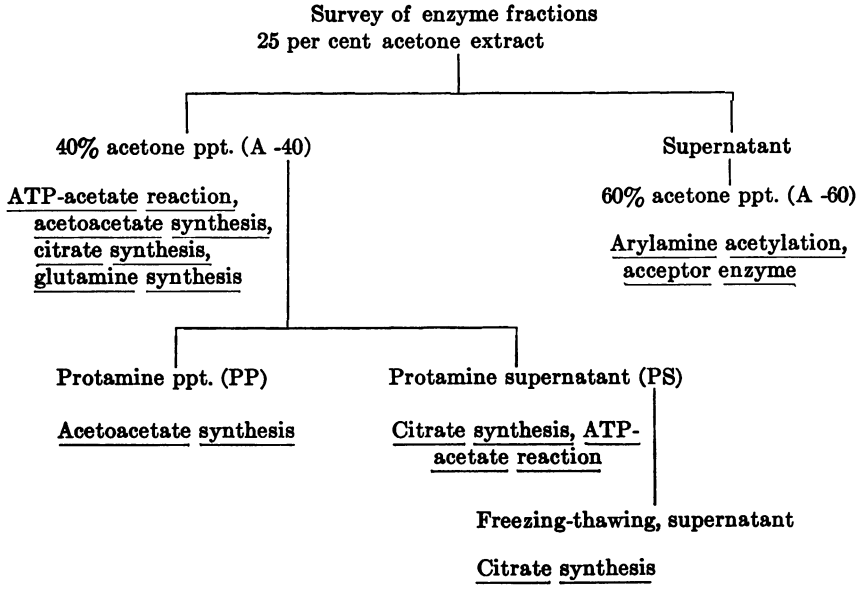

FIG. 4. Scheme of pigeon liver fractionation (13).

TABLE 2

Citric acid synthesis in dialyzed extract of E. coli (53)

ADDITIONS	CITRIC ACID SYNTHESIZED PER ML EXTRACT
	μM
None	0
Acetate, ATP	0.23
Acetate, ATP, coenzyme A	1.30
Acetyl phosphate	0.25
Acetyl phosphate, coenzyme A	4.0

All tubes contained: 1.0 ml of extract, 0.025 M oxalacetic acid, 0.016 M $NaHCO_3$, 0.02 M $MgCl_2$, and 0.01 M cysteine in a final volume of 2.5 ml. The concentrations of the additions were as follows: sodium acetate 0.05 M, sodium ATP 0.02 M, lithium acetyl phosphate 0.004 M, and coenzyme A 17 units.

it had started us on this trail. Now Novelli, studying the citric acid synthesis in *Escherichia coli* extract (53) found to our great surprise that in this system acetyl phosphate acts as an excellent acetyl donor for oxalacetate and indeed is more active than ATP and acetate, as shown in table 2. It was soon found that on addition of various microbial extracts to the acceptor fractions of pigeon liver, a

transfer system could be constructed linking acetyl phosphate with various acceptors, and alternating for ATP-acetate, the classical donor (36, 49). Through the work of Stadtman (61, 66), the microbial factor then was identified with an enzyme, "transacetylase" (64), first observed in *Clostridium kluyveri*. This enzyme catalyzes the reversible reaction between CoA and acetyl phosphate to form acetyl-CoA and inorganic phosphate. This was shown by exchange of acetyl bound phosphate with P^{32} and, in particular, by arsenolysis of acetyl phosphate (39, 66), both catalyzed by transacetylase but only in the presence of CoA. Table 3 presents data for P^{32} exchange.

The transacetylase reaction for acetyl phosphate is duplicated by another transacetylase reaction where pyruvate acts instead as the acetyl donor, forming acetyl-CoA and formic acid. Chantrenne in my laboratory (11) found that the system present in *E. coli* which exchanges formate with the carboxyl of pyruvate (70, 71) is a CoA dependent reaction, as shown in figure 5. It may

TABLE 3

CoA effect on interchange of acetyl-bound and P^{32}-labeled inorganic phosphate (66)

CoA	SUBSTANCE	RADIOACTIVITY
units per ml	*μM*	*cpm per μM*
0	inorganic P 98	2520
	acetyl P 18	7
40	inorganic P 91	2140
	acetyl P 17.2	1930

Conditions: cysteine, 0.01 M: tris(hydroxymethyl)aminomethane buffer (pH 8.1), 0.1 M; 1.2 mg of enzyme (Lot L), acid amonium sulfate fractions of pigeon liver extract precipitating at 43 to 86 per cent saturation; acetyl P, inorganic phosphate, and CoA (55 units per mg) as indicated to 1.0 ml total volume. After 30 minutes incubation (28 C), the inorganic P and acetyl P were separated, washed, and their P^{32} content determined.

also act (table 4) as an acetyl donor system for sulfanilamide acetylation. In a similar system, Ochoa and his group (28) have shown that pyruvate may act as acetyl donor for the acetyl group in citric acid. Ochoa's group (67, 69) also made the very important observation that the acetylation of oxalacetate is reversible and that under appropriate conditions citrate may act as acetyl donor.

In animal tissues, in yeast and also in some other microorganisms, as *Propionibacterium pentosaceum*, acetyl phosphate does not function as the precursor of acetyl-CoA. Indeed, acetyl phosphate appears not to be formed at all metabolically; it is a metabolic intermediary unknown to this type of organism. In that case, acetate is activated through a rather complex reaction involving ATP, a fact first discovered by Nachmansohn and Machado (49) in their studies of choline acetylation in brain extracts. The mechanism of this ATP-acetate reaction, or rather, as we now know of the ATP-*CoA*-acetate reaction, remained obscure for a long time. Recent studies in our laboratory (34, 35) have resolved the reaction sequence into two steps; the first step appears to be a pyrophos-

phorylation of CoA by ATP, the second an exchange of pyrophosphoryl (PP) for acetate (Ac):

$$ATP + CoA \cdot SH \rightleftharpoons CoA \cdot S \sim PP + AMP \quad (a)$$
$$CoA \cdot S \sim PP + Ac \rightleftharpoons CoA \cdot S \sim Ac + PP \quad (b)$$

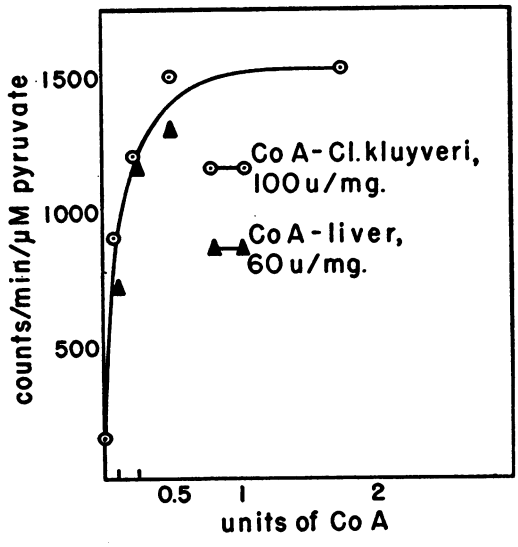

FIG. 5. Fixation of C^{14} formate in pyruvate as a function of CoA (11).

TABLE 4

Sulfanilamide acetylation with pyruvate as acetyl donor (11)

ENZYME MIXTURE	PYRUVATE	ACETYL PHOSPHATE	SULFANILAMIDE ACETYLATED
	μM	μM	μM
E. coli + liver	25		0
E. coli + liver + CoA	25		0.40
E. coli + liver + CoA		12	0.43
E. coli + liver + CoA	25	12	0.48

Freshly prepared, Dowex-1-treated *E. coli* extract was used. Samples were incubated in a total volume of 1 ml containing, if added, 0.3 ml of liver Fraction A-60 and 0.3 ml of *E. coli* extract. All samples contained 10 μM of cysteine, 0.1 M tris(hydroxymethyl)aminomethane (Commercial Solvents) buffer of pH 7.5, and 0.5 μM of sulfanilamide. The extract contained not more than $6 \cdot 10^{-4}$ M phosphate. 90 minutes incubation; temperature 37 C.

This mechanism emerged when, to our surprise, it was observed that not phosphate (*cf.* 44), but pyrophosphate, is the product of the reaction. In confirmation, adenosine monophosphate (AMP) and not ADP appeared as the reaction product, as was shown in balance experiments, such as represented in table 5. The pyrophosphate formation in this reaction had been obscured for a long time by the presence of a powerful pyrophosphatase, particularly in yeast

extracts, but also present in extracts of animal tissues. This mechanism has been verified for yeast and pigeon liver, and the reversibility of the sequence has been established. (33a, 35)

The exchange of a pyrophosphoryl for an acetyl group with the formation of acetyl~S·CoA indicates a pyrophosphoryl~S·CoA. The further identification of this important compound is in progress. The possibility of an S-P link as a

TABLE 5

Balance experiment showing accumulation of adenosine-5-phosphate and inorganic pyrophosphate as products of ATP-CoA-acetate reaction (35)

CoA	INCUBATION TIME	ATP	AMP	HYDROXAMIC ACID	PYROPHOSPHATE
units	*minutes*	*μM*	*μM*	*μM*	*μM*
0	0	29.0	0.2	0	0
	150	22.9	1.2	2.7	0
290	0	28.7	2.8	0	0
	150	0.4	19.7	32.5	24.9

Each vessel contained: 29 μM ATP, 250 μM acetate, 860 μM NH_2OH (pH 6.5), 80 μM glutathione, 160 μM potassium fluoride, 640 μM tris(hydroxymethyl)aminomethane buffer (pH 7.4), 32 μM $MgCl_2$, and 0.32 ml of the yeast enzyme in 3.2 ml total volume.

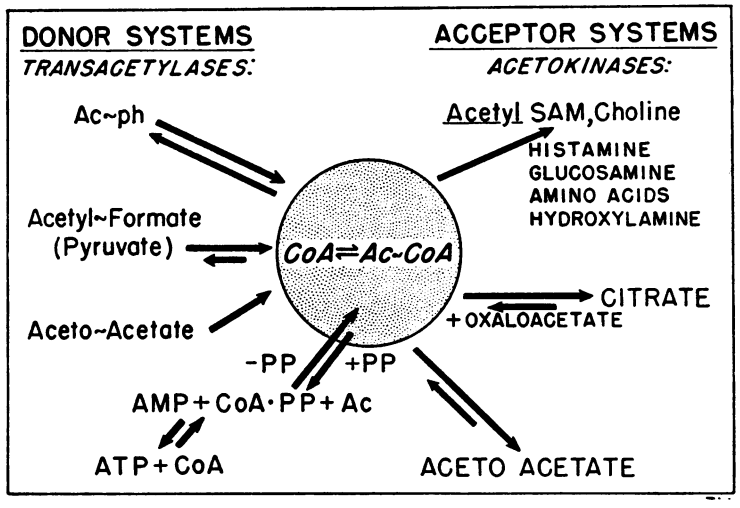

FIG. 6. Map of acetyl transfer "territory".

"bridge" between ATP and acetyl-CoA appears of rather general significance as a procedure in biosynthesis.

Making now an attempt from all these observations to draw a map of the acetyl transfer "territory", the following scheme in figure 6 presents itself: This scheme indicates the function of CoA as a general acetyl carrier, shuttling between the donor systems, the "transferases", and the acceptor-specific enzymes, "acetokinases"; this terminology seems to be indicated by the obvious analogy

of the acetyl donor function of acetyl-CoA through *aceto*kinases to the phosphoryl donor function of ATP through *phospho*kinases. Such a nomenclature duly stresses the general uniformity of biochemical procedure.

Although this scheme covers the basic function of CoA, it has become clear, during the last few years, that CoA acts as a carrier not only for acetyl, but much more generally for other acyl groups. Chantrenne (10) made the important discovery that hippuric acid synthesis by way of benzoic acid, ATP, and glycine

TABLE 6

Coenzyme A-catalyzed acetyl transfer reactions

TYPE OF REACTION	ENZYME SYSTEMS, EXTRACTS	REFERENCE
Donor Systems:		
ATP-acetate-CoA → acetyl-CoA	pigeon liver, yeast	13, 31, 40, 42
phospho-transacetylation, ac-P + CoA ⇌ ac-CoA + P	*Clostridium kluyveri, Escherichia coli*	62, 64, 66
formo-transacetylation, pyruvate + CoA ⇌ ac-CoA + formate	*Escherichia coli*	11, 28
transacetylase:		
ac-CoA + butyrate ⇌ butyryl-CoA + acetate	*Clostridium kluyveri*	63
acetoacetate	pigeon liver	60
citrate in reverse	pigeon liver	69
acetaldehyde + CoA + DPN^+ → ac-CoA + DPNH + H^+	glyceraldehyde dehydrogenase	22
	Escherichia coli	55
	Clostridium kluyveri	9
pyruvate + CoA + DPN^+ → ac-CoA + DPNH + CO_2 + H^+	*Escherichia coli, Streptococcus faecalis*	27
	heart and muscle	57
Acceptor Systems, Aceto-kinases:		
aromatic amines	pigeon liver	32
choline	rat brain	49
histamine	pigeon liver	46
amino acids	*Clostridium kluyveri*	64
glucosamine	pigeon liver	15
Condensation:		
acetoacetate	pigeon liver	60, 65
citric acid	pigeon liver, yeast, heart	53, 54, 68
pyruvate	*Escherichia coli*	11, 41, 70, 72

(45) is catalyzed by an enzyme system requiring CoA. This indicates that CoA acts as benzoyl carrier and that presumably benzoyl-CoA is intermediary.

More important even, appear the recent observations by the Green group (19) as well as the Ochoa group (23) that the oxidation of ketoglutarate occurs by way of succinyl-CoA. The possibility of a succinyl transfer "territory" is forecast by recent work of Shemin (58), who presents rather interesting evidence for a succinyl-"X" being an intermediary in the formation of the pyrrole rings in hemin. The "X" in Shemin's formulation presumably represents CoA.

A survey of the presently known CoA linked reactions is presented in tables 6 and 7.

The connecting of pantothenic acid deficiency phenomena with functional analysis has been of considerable help all through the work on CoA. More recently, we used this approach for a study of lipid synthesis as related to CoA, which I shall now discuss in some detail.

One of the earlier observations on the nutritional effects of pantothenic acid deficiency had connected the adrenal cortex with the deficiency, in particular, through the work of Morgan (22a, 47), who first observed a degeneration of the adrenal cortex with pantothenic acid deficiency. This observation seemed to relate steroid hormone formation to CoA. Isotope work mainly by Bloch (5, 6)

TABLE 7

Coenzyme A-catalyzed acyl transfer reactions

TYPE OF REACTION	ENZYME SYSTEMS, EXTRACTS	REFERENCE
Succinyl Transfer		
Donor System:		
ketoglutarate + DPN^+ + CoA → succinyl-CoA + DPNH + H^+	heart muscle	19, 23, 56
ATP-succinate	*Escherichia coli*	31
Acceptor System:		
heme synthesis	red cells hemolysate	58
Benzoyl Transfer		
hippuric synthesis	rat liver	10, 45
Stearyl Transfer		
phospholipid synthesis	rat liver	30
Complex Reaction Systems:		
fatty acid synthesis	*Clostridium kluyveri* extracts	3, 64
butyrate oxidation	*Clostridium kluyveri* extracts	24
fatty acid oxidation	liver homogenate	12
steroid and fat synthesis	resting yeast cells	25
	liver slices	26

had shown that "active acetate", identified now as acetyl-CoA, is involved in the synthesis of steroids as well as fatty acids. Dr. Klein (26), in my laboratory, obtained evidence for a connection between the CoA content and lipid synthesis, in particular ergosterol synthesis in yeast (25). These results are shown in table 8. Using pantothenic acid-deficient rats, he made similar observations connecting the CoA content of the liver with the synthesis of cholesterol and fatty acids. The CoA content in the liver appeared to be the most important factor, more important than the general appearance of the animal. Even if an animal is rather deficient, with typical deficiency signs, but still contains a relatively high level of CoA in the liver, its lipid synthesis is found to be normal, while if CoA drops to about 50 units per gram, the lipid synthesis begins to decrease.

Rather suggestive observations were made, furthermore, by using the pantothenic antimetabolite, pantoyl-tauryl-anisidide. The compound was tried on liver slices for its effect on incorporation of radioactive acetate into fatty acids. The antimetabolite inhibited lipid synthesis, but respiration showed no decrease. From recent experiments it appears that such antagonism is observed only with livers of low CoA. This effect in some experiments could be counteracted with pantethine (LBF) while pantothenic acid was inactive. This result seems to correlate with earlier observations by Olson and Kaplan (54a) that in tissue slices pantothenic acid is a rather poor precursor for CoA. We are, unfortunately, with this type of experiment still far from a characterization of enzyme

TABLE 8

Lipid synthesis with CoA-poor and CoA-rich, resting yeast (25)

NO.	PANTOTHENIC ACID ADDED	CoA PER GRAM CELLS	SYNTHESIS PER GRAM CELLS	
			Steroid	Total lipid
	μg/ml	*units*	*mg*	*mg*
1	0	37	−0.2	
	0.02	67	1.2	
2	0	47	0.2	2.4
	10	280	2.5	12.7
3	0	60	0.4	1.0
	10	310	1.8	8.6
4	0	57	0.2	1.2
	10	300	1.7	9.6
5	0	60	1.8	
	10	165	6.2	

Identical pantothenic acid-deficient yeast samples were incubated with and without pantothenic acid for 2–3 hours and washed. Subsequently, the yeast samples were incubated for 16 hours in an acetate-phosphate medium and analyzed for CoA, total lipid and steroid.

systems performing synthesis of fatty acids and steroids from acetyl-CoA. With the synthesis of lower fatty acid, however, a very important beginning has been made by Barker and Stadtman (3), using extracts of *Clostridium kluyveri*.

In the field of phospholipid synthesis, Kornberg's recent work (29) is very promising. He showed that stearyl-CoA may form in liver extracts from ATP, CoA, and stearate in a similar fashion as we have shown for acetyl-CoA to form from ATP, CoA, and acetate, presumably by way of pyrophosphoryl-CoA. In the same extract, stearyl-CoA is linked through another enzyme to α-glycerophosphate completing the phospholipid synthesis.

The work discussed in the last paragraphs still represents a preliminary approach to an understanding of the functioning of CoA in the synthesis of larger molecules, such as steroids or long-chain fatty acids. However, the identity of

acetyl-CoA with the 2-carbon fragment of the isotope workers makes it obvious that wherever acetate is incorporated, CoA must be involved in some manner. In addition, we now recognize that CoA functions as an acyl carrier, not only for acetic acid, but much more generally for a number of carboxylic acids. This functioning increases the field of action of CoA considerably. A complete mapping of the territory, therefore, will take some time, and a good deal of new information on the intermediary reactions may still be expected from future work with this coenzyme.

In general, I feel that it is encouraging to realize that we are slowly learning to deal more intelligently with the working mechanisms of metabolic catalysts. It is one of the most satisfying results of biochemical research when it appears that biochemical phenomena start to group themselves in such a manner as to explain, at least in certain areas, the seemingly so complex biochemistry of the whole organism.

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